Prunes and Biotechnology

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Dried Plums and Biotechnology

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To acknowledge

- The work of Chris Dardick and Ralph Scorza and the USDA/ARS for their work on plums in many areas

Outline

- What you would like from biotechnology ?
- Transgenic Plums
- Pitless plums
- Embryo rescue
- Disease resistant transgenics
- Marker Assisted Selection
- FasTrack rapid breeding system
- Genome Sequencing
- Future Genetic Selection Strategies

Pests, diseases and breeding objectives for plums

- Aphids
- Codling moth
- PPV
- Phytophthora
- Canker
- Plum decline
- Brown rot
- Rust
- Russet scab
- Potassium deficiency
- Fruit Quality:
  - texture, flavour, colour, storability, high sugars
- Fruit appearance
- Adaptability
- Pest & disease resistance
- Growth habit
- Flowering
- Cropping
- Novelty

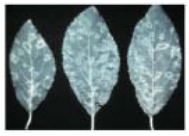
PPV incidence

- Restricted Distribution
  - Albania, Austria, Canada, Cyprus, Czech Republic, France, Italy, Luxembourg, Moldova, Norway, Portugal, Southern Russia, Slovenia, Spain, Syria, Turkey, Ukraine, United Kingdom, United States
- Widespread
  - Bulgaria, Croatia, Germany, Greece, Hungary, Poland, Romania, Slovakia
- Introduced, Established
  - Azores, Bosnia-Herzegovina, Egypt, Former USSR including Central Asia, India, Lithuania
- Introduced, Presumably Eradicated
  - Belgium, Netherlands, Switzerland
- Present Status Unknown
  - Chile, Denmark

- Modified from: Levy et al. 2000. Plum Pox Potyvirus Disease of Stone Fruits. American Phytopathological Society[4]

PPV Resistant Transgenic plums

- Coat protein mediated resistance
- Same mechanism as used in Papaya
- Or EIF4e from peach to confer resistance



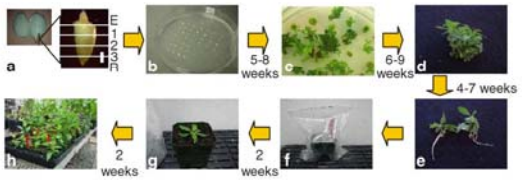
Pitless plums

- Molecular biologists Chris Dardick and Ann Callahan and Prunus breeder Ralph Scorza are looking for the genes that control pit formation, and lignin production, to breed or engineer pitless plums

Embryo rescue for breeding seedless plums

- Embryo rescue allows the germination of viable embryos from non-viable seeding systems
- Good for breeding seedless plants
- And for interspecific hybrids

Transformation of plums



Suppression of ACO for reduced ethylene production

- 18 lines from transformed BlueByrd showed some evidence of reduced softening, but also reduced sugar
- Aiming to slow ripening/softening for fresh fruit shipping and long life product

Gastrodia Anti Fungal Protein

- Anti-fungal protein from the orchid *Gastrodiaelata*
- When expressed in transgenic plums gives increases resistance to Phytophthora root rot and root-knot nematode
- May be a useful strategy for rootstock improvement

Marker Assisted Selection in Plum Breeding

Targets for MAS

- Clingstone/Freestone
- Sugar and acid balance
- Texture
- Flowering time
- Ethylene/ripening

RosBREED

Traditional Breeding approach

- Select two good cultivars and make crosses

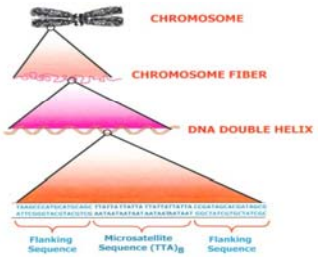
- Select seedlings from a cross

  perform pathology / chillingrequirement /fruit quality trials
- Trees have long breeding cycles (5-20 years)

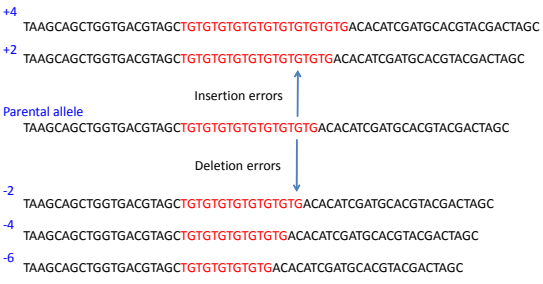
Marker Assisted Breeding

- To identify and use markers to assist with the selection of economically desirable phenotypes
- To increase the speed of selection in breeding program
- To increase the number of seedlings tested
- To allow geneticselection of many traits at once early in breeding program

Microsatellites



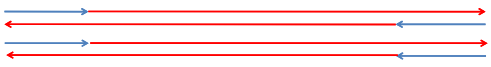
How Microsatellites become polymorphic to create alleles



PCR Analysis of Microsatellites

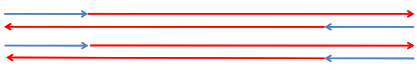
Allele 1

TAAGCAGCTGGTGACGTAGCTGTGTGTGTGTGTGTGTGTGACACATCGATGCACGTACGACTAGC
TAAGCAGCTGGTGACGTAGCTGTGTGTGTGTGTGTGTGTGACACATCGATGCACGTACGACTAGC



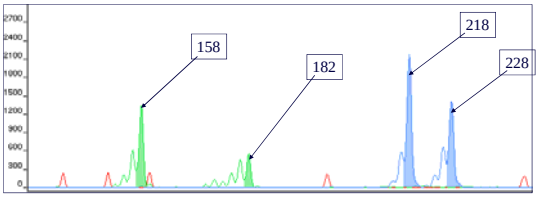
Allele 2

TAAGCAGCTGGTGACGTAGCTGTGTGTGTACACATCGATGCACGTACGACTAGC
TAAGCAGCTGGTGACGTAGCTGTGTGTGTACACATCGATGCACGTACGACTAGC

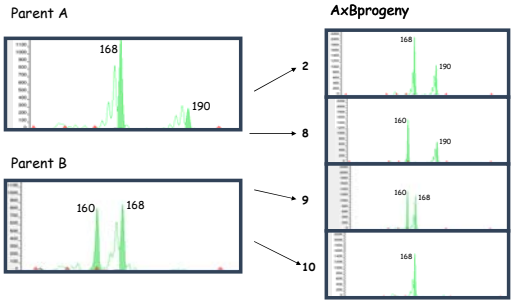


Microsatellite Analysis

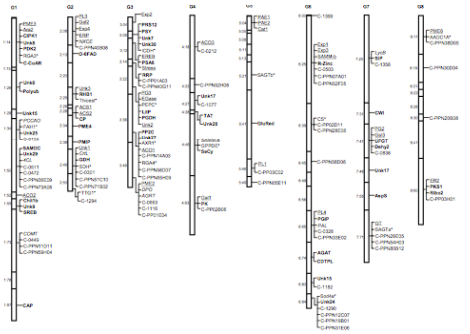
- PCR with fluorescent primers
- Analysis by capillary electrophoresis
- Detection and scoring of alleles



Detection of alleles in a cross between A and B



Prunus Fruit Quality Gene Map



FasTrack rapid breeding of plums

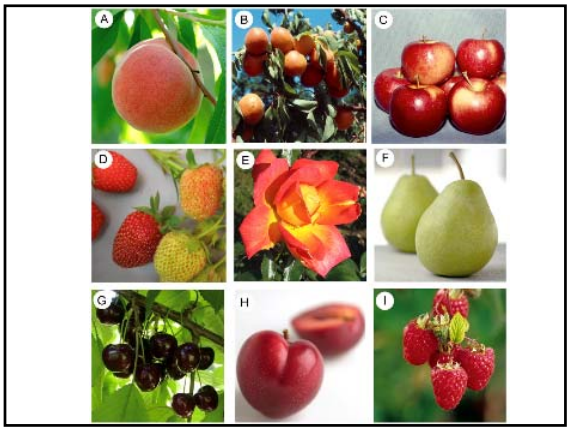
- PtFT1 – flowering time gene from Poplar
- Flowering plants were also characterized by the precocious production of lateral branches upon which flowers were produced.
- Following pollination, fruit developed and ripened normally and formed viable seed.
- Plants flower and set fruit in under one year from germination

FasTrack rapid breeding of plums

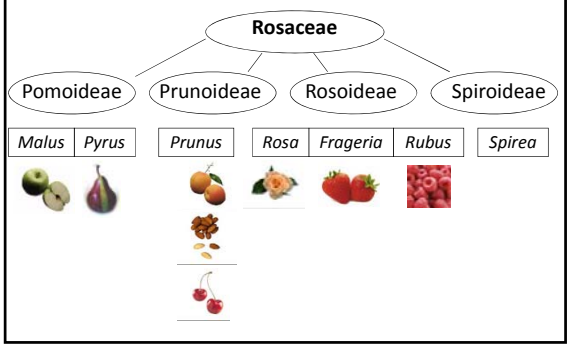
- Seeds harvested from first generation PtFT1 transgenic plums were germinated, and these produced flowering second generation plums within four months and segregated into PtFT1 transgenic lines and non-flowering, non-transgenic lines.
- Ectopic expression of PtFT1 reduced plum generation time from three to seven years to one year.
- A plum genetic improvement program has been initiated to rapidly incorporate desired traits into plum with the eventual selection of non-transgenic improved germplasm and varieties.

FasTrack Breeding gives you:

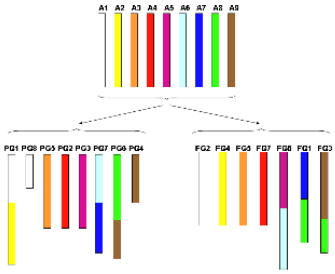
- Breeding cycle reduced to One Year
- Together with genetic selections will allow breeding of highly selected cultivars in 5-6 years, not 25-30 years
- The breeding intermediate is transgenic
- But the last cycle breeds out the early flowering transgene
- So the final variety is non-transgenic and flowers after the normal 3-5 year juvenile period, making it completely equivalent to a conventional breeding product



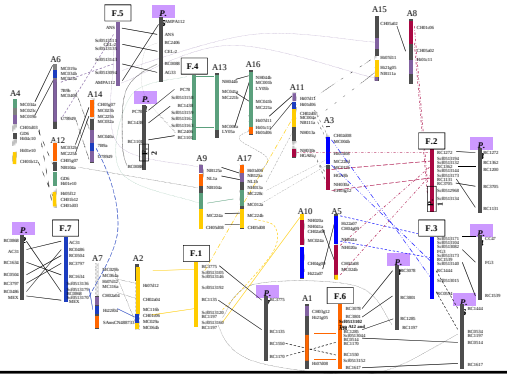
Similarity among the Rosaceae



Strawberry and Prunus: Ancestral genome



Apple, Peach, Strawberry



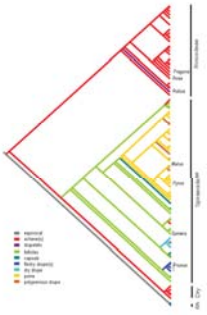
Similarity among Prunus species

- Plums
- Peaches/Nectarines
- Apricots
- Cherries
- Almonds

- All have 8 equivalent chromosomes
- Interspecific crosses are possible
- Can cross with wild material
- However often very closely related genetic material used in breeding programs

Using the similarity among *Prunus* species

- Many traits have related genetics
- Genes from one system can cause the same effects in related species
- Genome sequences will be very similar



Fruit and Nut Tree Genomics Ongoing genome sequencing

- Published
  - Grape (2 variants, more in progress)
- First drafts about to be published
  - Apple – diploid Golden Delicious
  - Peach – doubled haploid Lovell
  - Strawberry – diploid *F. vesca*
- Projects in progress or planned
  - Citrus, Mango, Banana, etc
  - Hazelnut, walnut, chestnut, cacao, coffee
- Lots or other species in need of genomics and genetics

Genomics of the genus Prunus

- Peach genome sequence completed and released in April 2010
- 20 peach varieties sequenced by June 2010
- Genome annotation completed to the first draft level
- New genetic marker chips available in 2011
- Plum genome sequence data generated by August 2010
- Expecting to assemble using peach genome framework
- Gene annotation and release during 2011
- SNP data during 2011

Whole Genome Selection for Breeding

- 10 000 SNP markers will be possible for plum during 2011
- Will need variety resequencing and enough samples for economically viable SNP analysis
- This will provide the basis for whole genome selection in breeding programs

Summary

Transgenic Plums	Yes
Pitless plums	Almost
Embryo rescue	Yes
Disease resistant transgenics	Preliminary
Marker Assisted Selection	Yes
FasTrack rapid breeding system	Yes
Genome Sequencing	Soon
Future Genetic Selection Strategies	Soon